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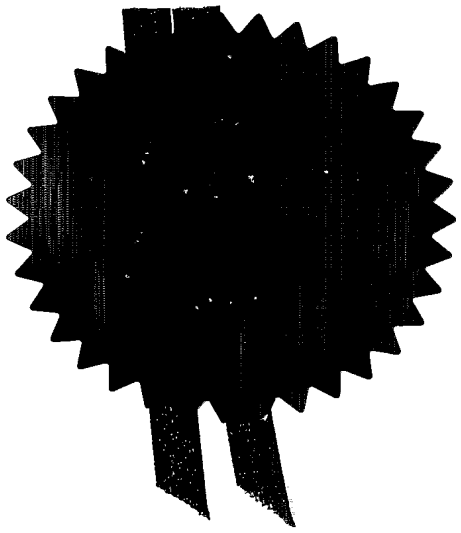

Signed Andrew Gersey

Dated 29 December 2004

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1. Your reference — 46313.GB01/NT

2. Patent application number *(The Patent Office will fill in this part)* — 05 DEC 2003 — 0328319.9

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

Cambridge Biotechnology Limited
PO Box 230
Cambridge
CB2 1XJ
UNITED KINGDOM

Patents ADP number *(if you know it)* 08206761002

If the applicant is a corporate body, give the country/state of incorporation — UNITED KINGDOM

4. Title of the invention

Improved Synthesis of 2-substituted Adenosines

5. Full name, address and postcode in the United Kingdom to which all correspondence relating to this form and translation should be sent

Reddie & Grose
16 Theobalds Road
LONDON
WC1X 8PL

Patents ADP number *(if you know it)* 91001

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application *(If you know it)*	Date of filing *(day/month/year)*

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Number of earlier application	Date of filing *(day/month/year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

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YES

Patents Form 1/77

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Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	0
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11. I/We request the grant of a patent on the basis of this application.

Signature: Reddie e Grose

Date: 4 December 2003

12. Name and daytime telephone number of person to contact in the United Kingdom: Neil Thornton 01223 360350

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Improved Synthesis of 2-Substituted Adenosines

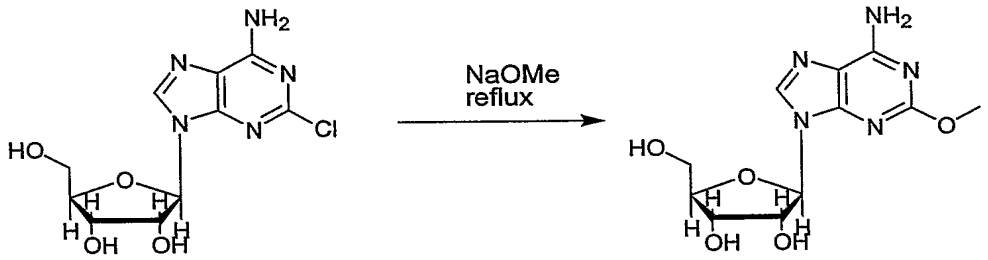
This invention relates to synthesis of 2-substituted adenosines, such as spongosine (2-methoxyadenosine) and to synthesis of intermediates for use in the synthesis of such compounds.

The natural product spongosine was first isolated from a sponge, *Cryptotethia crypta,* collected off the Florida coast in 1945 (Bergmann and Feeney, J.Org. Chem. 1951, 16, 981; Ibid 1956, 21, 226). Spongosine was considered an unusual nucleoside in that it was not only the first methoxypurine to be found in nature but also one of the first O-methyl compounds to be isolated from animal tissues.

Several syntheses of spongosine have been previously reported. One of the first of these to be published was by Bergmann and Stempien (J. Org. Chem. 1957, 22, 1575) in which spongosine was formed via coupling of chloromercuric 2-methoxyadenosine to 2,3,5-tri-O-benzoyl-D-ribofuranosyl chloride. This simple coupling reaction provided a crude yield of spongosine of 31% which was then recrystallised from hot water to provide spongosine which exhibited a melting point of 191-191.5°C and an optical rotation of -43.5° (NaOH).

A variation on this theme was employed by Ojha *et al.* (Nucleosides and Nucleotides (1995, 14, 1889) who initially coupled 2-ethylthioadenine with a suitably protected ribose. Subsequent adjustments of the protecting groups and oxidation gave a substrate which was reacted with sodium methoxide to yield spongosine in a yield of 87% for the final step. The purity of the target spongosine after column chromatography clean up, was proved by both elemental analysis and melting point (189-190°C).

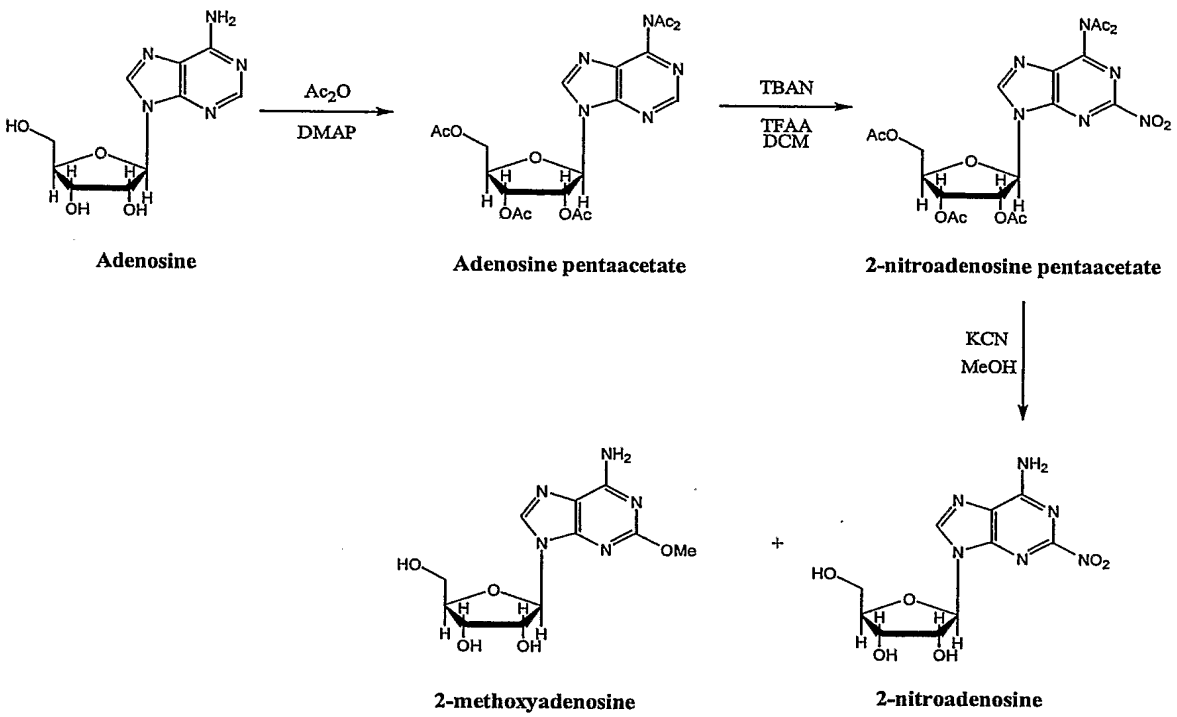
One of the most common methods of preparation of spongosine is via displacement of a 2-substituted chlorine atom by methoxide:



This methodology has been successfully applied by a number of groups to provide spongosine in varying yields and purity: Schaeffer *et al.*; J. Am. Chem. Soc. 1958, 80, 3738 (35% yield, mpt. 190-192°C); Bartlett *et al.*; J. Med. Chem. 1981, 24, 947 (yield and purity not quoted), Sato *et al.*; Synth. Proceed. Nucleic Acid Chem. 1968, 1, 264. However, this method suffers from the disadvantage that the 2-chloroadenosine starting material is difficult to synthesise and expensive.

Spongosine was reported by Cook *et al.* (J. Org. Chem. 1980, 45, 4020) as a by-product in the methylation reaction of isoguanosine by methyl iodide. Both the desired 1-methylisoguanosine and the spongosine were obtained in poor crude yields (19 and 30% respectively). The crude spongosine fragment was first purified by column chromatography on silica gel (eluent: chloroform/methanol) and then recrystallised from water to provide a sample which melted between 189-192°C (7% yield pure).

Paymaneh *et al.* (Tetrahedron Letters 41 (2000) 1291-1295) and Wanner *et al* (Bioorganic & Medicinal Chemistry Letters 10 (2000) 2141-2144) describe formation of spongosine as a significant by-product in the synthesis of 2-nitroadenosine by treatment of 2-nitroadenosine pentaacetate with potassium cyanide in methanol. The 2-nitroadenosine was obtained in only 10% yield, and spongosine in 47% yield (Paymaneh *et al.*). The 2-nitroadenosine pentaacetate was produced by nitration of adenosine pentaacetate with tetrabutylammonium nitrate/trifluoroacetic anhydride (TBAN/TFAA), and (in Wanner *et al.*) the adenosine pentaacetate was formed by treatment of adenosine with acetic anhydride and DMAP:



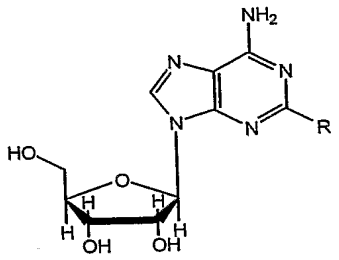
Synthesis of spongosine (2-methoxyadenosine) according to Wanner *et al.*

A disadvantage of this method is that the spongosine is not produced in high yield or purity. A further disadvantage of the method is that it involves use of the toxic reagent potassium cyanide. It is desired, therefore, to provide alternative methods of synthesis of spongosine, and to improve the yield and purity of the spongosine produced.

We have appreciated that one of the principal reasons that spongosine is not produced in high yield and purity by the method of Paymaneh *et al.*, and Wanner *et al.*, is that TBAN contaminates the 2-nitroadenosine pentaacetate and interferes with the subsequent methoxylation and deprotection of this compound. This is also the case if tetramethylammonium nitrate (TMAN) is used instead of TBAN.

According to a first aspect of the invention there is provided a method of synthesising a 2-substituted adenosine of formula I, which comprises: nitrating adenosine pentaacetate using TBAN or TMAN to produce 2-nitroadenosine pentaacetate; reducing the amount of

TBAN or TMAN contaminating the 2-nitroadenosine pentaacetate; and then producing the 2-substituted adenosine from the 2-nitroadenosine pentaacetate:



I

wherein R = C_{1-6} alkoxy (straight or branched), a phenoxy group (unsubstituted, or mono-, or di-substituted by halo, amino, CF_3-, cyano, nitro, C_{1-6} alkyl, or C_{1-6} alkoxy) or a benzoyl group (unsubstituted, or mono-, or di-substituted by halo, amino, CF_3-, cyano, nitro, C_{1-6} alkyl, or C_{1-6} alkoxy).

Preferably R is methoxy, ethoxy, propoxy, butoxy, pentyloxy, hexyloxy, phenoxy, or benzoyl.

Removal of TBAN and TMAN is particularly difficult because these compounds are amphiphilic and so cannot be removed by aqueous work-up.

It has surprisingly been found that an effective method of reducing the amount of TBAN and TMAN contaminant is trituration of the 2-nitroadenosine pentaacetate with isopropanol, followed by washing with water. This can significantly improve the purity and yield of the spongosine or other 2-substituted adenosine product.

There is also provided according to the invention a method of reducing the amount of TBAN or TMAN contaminating 2-nitroadenosine pentaacetate formed by nitration of adenosine pentaacetate with TBAN or TMAN, which comprises triturating the 2-

nitroadenosine pentaacetate with isopropanol and washing the triturated 2-nitroadenosine pentaacetate with water to reduce the amount of TBAN or TMAN.

There is further provided according to the invention 2-nitroadenosine pentaacetate produced by such methods.

Preferably nitration is carried out using TBAN or TMAN with trifluoroacetic anhydride (TBAN/TFAA, or TMAN/TFAA). Preferably the TBAN/TFAA or TMAN/TFAA is in dichloromethane (DCM). A preferred method of nitration of adensoine pentaacetate is described in the example below.

2-nitroadenosine pentaacetate may be converted to the 2-substituted adenosine by deprotecting the 2-nitroadenosine pentaacetate and reaction with a suitable anion (for example a C_{1-6} alkoxide anion, or a phenoxide anion). To synthesise spongosine this may be achieved by reaction with potassium cyanide and methanol as detailed in Paymaneh *et al.,* and Wanner *et al.* However, it is preferred that less toxic sources of the methoxide anion are used. Preferred sources are MeOH/NaOMe, MeOH/n-BuLi, MeOH/NaOH, or MeOH/NaH. A preferred method of conversion of 2-nitroadenosine pentaacetate to spongosine is described in the example below. It is believed that other 2-substituted adenosines may be synthesised by treatment of the 2-nitroadenosine pentaacetate with an appropriate C_{2-6} alcohol, or a phenol, and sodium hydroxide.

According to a further aspect of the invention there is provided a method of synthesising spongosine which comprises treating 2-nitroadenosine pentaacetate with MeOH/NaOMe, MeOH/n-BuLi, MeOH/NaOH or MeOH/NaH to form spongosine.

There is also providing according to the invention a method of synthesising a 2-substituted adenosine of formula I, excluding spongosine, which comprises deprotecting 2-nitroadenosine pentaacetate, and reaction with a C_{2-6} alkoxide anion, or a phenoxide anion. It is believed that this may be achieved by reaction with an appropriate C_{2-6} alcohol, or a phenol, and sodium hydroxide.

Methods of the invention may further comprise converting adenosine to adenosine pentaacetate. This may be achieved by the method detailed by Paymaneh *et al.*, and Wanner *et al.* However, we have appreciated that adenosine pentaacetate is produced only in low yield and purity using this method, and that the tetra-acetylated precursor is present as a major by-product.

We have found that the yield and purity of the 2-substituted adenosine product may be improved if methods of the invention further comprise acylating adenosine to form an O-tri-acetyl and/or tetra-acetyl derivative of adenosine, isolating the derivative(s), and acylating the isolated derivative(s) to produce the adenosine pentaacetate intermediate.

According to a further aspect of the invention there is provided a method of synthesising adenosine pentaacetate or a 2-substituted adenosine of formula I, which includes the following steps: acylating adenosine to form an O-tri-acetyl and/or tetra-acetyl derivative of adenosine, isolating the derivative(s), and acylating the isolated derivative(s) to produce adenosine pentaacetate.

The O-tri-acetyl and/or tetra-acetyl derivative can be isolated using column chromatography.

We have also found that the fifth acetate group of the penta-acetyl compounds is labile, and this results in decomposition of these compounds to tetra-acetyl compounds. For example, we purified adenosine pentaacetate by column chromatography, but there was evidence to suggest that the compound decomposed during this process. Attempts to recrystallise this compound were not successful and it was amorphous rather than crystalline in nature.

We have appreciated that the yield and purity of the 2-substituted adenosine product may be improved if methods of the invention alternatively or additionally further comprise

washing the adenosine pentaacetate intermediate to reduce the amount of contaminating adenosine tetraacetate before nitrating the washed adenosine pentaacetate.

According to a further aspect of the invention there is provided a method of synthesising adenosine pentaacetate or a 2-substituted adenosine of formula I, which includes the following steps: acylating adenosine or an acylated derivative of adenosine to form adenosine pentaacetate; and washing the adenosine pentaacetate to reduce the amount of contaminating adenosine tetraacetate.

To wash the adenosine pentaacetate, it is preferably dissolved in chloroform and washed with acetic acid solution (preferably 1M).

It is thought that 2-nitroadenosine pentaacetate may be toxic. Thus, it may be desirable to ensure that a 2-substituted adenosine produced from 2-nitroadenosine pentaacetate is contaminated with as little 2-nitroadenosine pentaacetate as possible. According to the invention this may be achieved by converting the 2-nitroadenosine pentaacetate to 2-chloroadenosine pentaacetate before converting the 2-chloroadenosine pentaacetate to the 2-substituted adenosine.

Conversion of 2-nitroadenosine pentaacetate to 2-chloroadenosine pentaacetate may be achieved by chlorinating the 2-nitroadenosine pentaacetate with a suitable chlorinating reagent, such as ammonium chloride.

According to a further aspect of the invention there is provided a method of synthesis of a 2-substituted adenosine of formula I which comprises converting 2-chloroadenosine pentaacetate to the 2-substituted adenosine.

There is also provided according to the invention use of penta-acetylated 2-chloroadenosine in the synthesis of a 2-substituted adenosine.

2-chloroadenosine pentaacetate may be converted to the 2-substituted adenosine by deprotecting the 2-chloroadenosine pentaacetate and reaction with a suitable anion (for example a C_{1-6} alkoxide anion, or a phenoxide anion). To synthesise spongosine this may be achieved by reaction with potassium cyanide and methanol as detailed in Paymaneh *et al.*, and Wanner *et al.* However, it is preferred that less toxic sources of the methoxide anion are used. Preferred sources are MeOH/NaOMe, MeOH/n-BuLi, MeOH/NaOH, or MeOH/NaH. It is believed that other 2-substituted adenosines may be synthesised using an appropriate C_{2-6} alcohol, or a phenol, and sodium hydroxide.

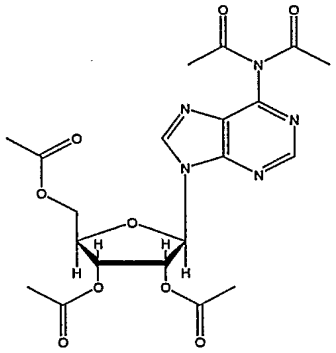
There is also provided according to the invention a 2-substituted adenosine of formula I, or an intermediate for use in synthesis of a 2-substituted adenosine of formula I, produced by a method of the invention.

Methods of the invention can be used to synthesise 2-substituted adenosines in high yield and purity. For example, we have been able to synthesise spongosine which is >96% pure.

Embodiments of the invention are now described by way of example only with reference to the accompanying Schemes 1 and 2 which show preferred methods of synthesis of 2-methoxyadenosine (spongosine).

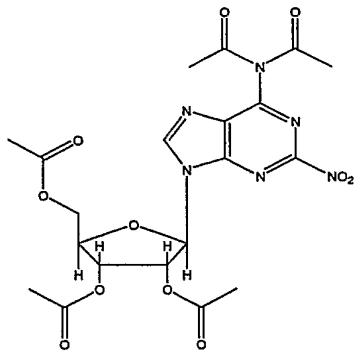
Example

Preparation of Adenosine Pentaacetate



To a solution of adenosine (1.0g, 3.74mmol) in acetic anhydride (10mL) was added sodium hydride (60% in mineral oil, 0.9g, 22.5mmol) and the mixture was heated at 110^0C for 20h. Reaction mixture was allowed to cool to room temperature, then poured onto ice/$NaHCO_3$ (250mL). EtOAc (150mL) was added and organic phase washed with water (3 x $100cm^3$), dried ($MgSO_4$) and the solvent removed in *vacuo*. The crude product was purified by silica gel chromatography (silica gel 60), eluting with EtOAc:Heptane (1:1), increasing to EtOAc to give the desired product (0.6g, 31%).

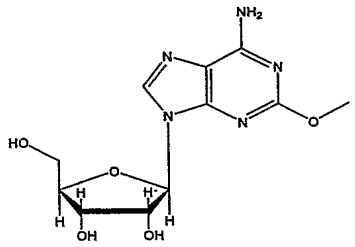
Preparation of 2-Nitro-Adenosine Pentaacetate



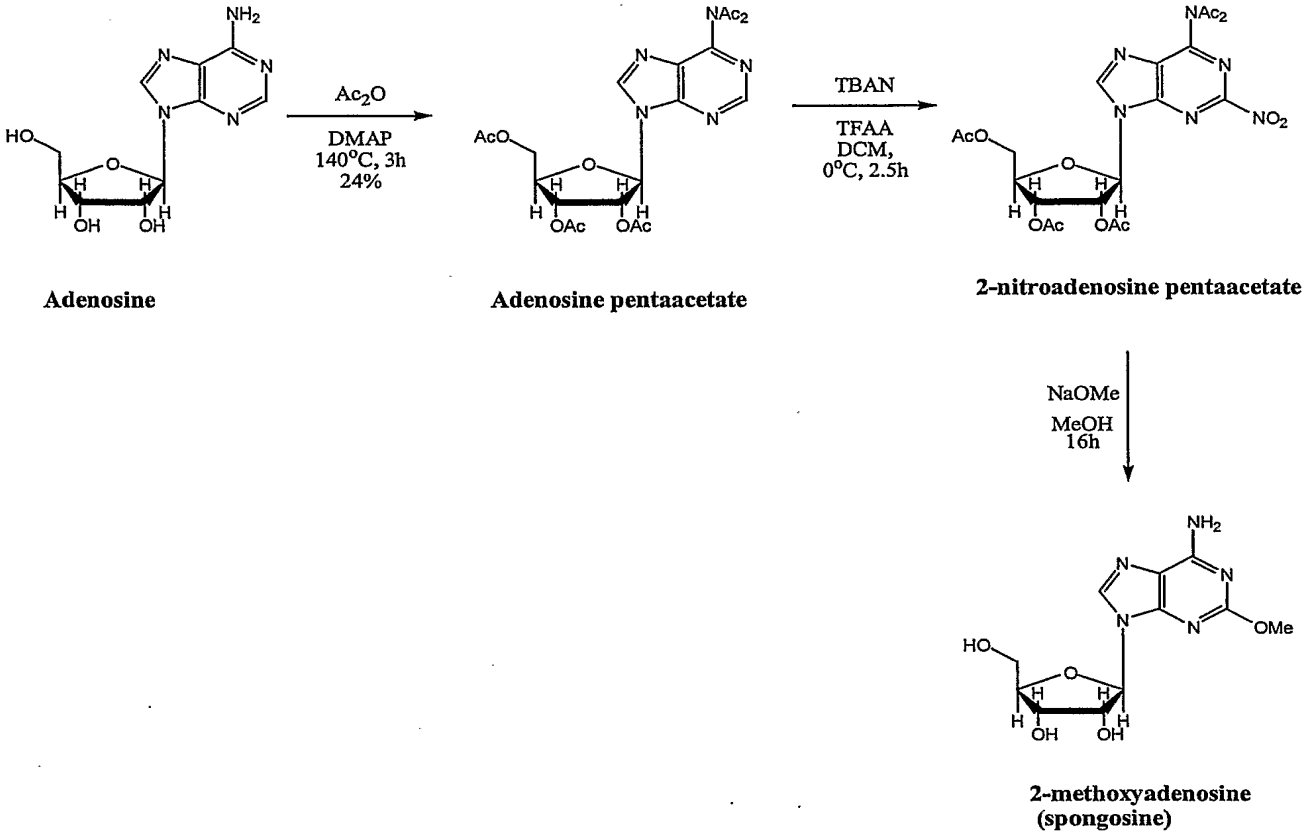
To a suspension of tetramethylammonium nitrate (642mg, 4.72mmol) in DCM (10mL) was added trifluoroacetic anhydride (0.68mL, 4.72mmol) and the resulting suspension stirred at room temperature for 1h before cooling to 0 °C. A solution of adenosine

pentaacetate (1.50g, 3.14mmol) in DCM (10mL) was added and the solution was allowed to warm to room temperature over 2.5h. The crude product was then washed with brine, dried ($MgSO_4$) and the solvent was removed *in vacuo* to give the target product as a pale brown solid foam (1.36g, 83%).

Preparation of 2-Methoxy Adenosine (Spongosine)



To a solution of 2-nitro-adenosine pentaacetate (275mg. 0.53mmol) at room temperature was added NaOMe (71mg, 1.3mmol) and the mixture stirred for 3h. Ammonium chloride (70mg, 1.3mmol) was added and the reaction mixture concentrated in *vacuo* to give a yellow oil. The crude product was purified by silica gel chromatography, eluting with EtOAc, increasing to EtOAc:MeOH (15:1) and then recrystallisation from isopropanol to give the target product as a white solid (70mg, 47%).



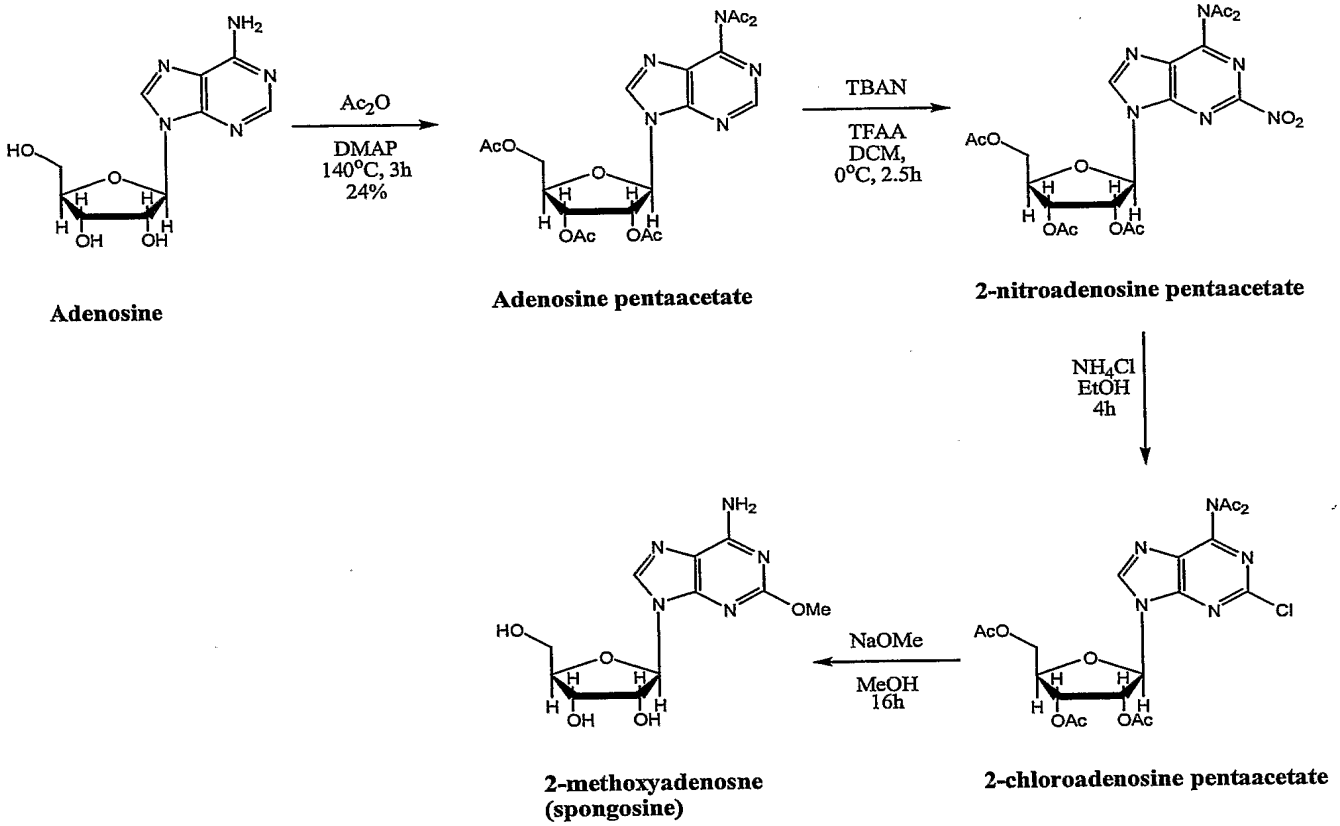
Adenosine

Adenosine pentaacetate

2-nitroadenosine pentaacetate

2-methoxyadenosine (spongosine)

Scheme 1



Adenosine

Adenosine pentaacetate

2-nitroadenosine pentaacetate

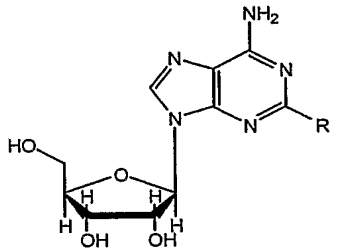
2-chloroadenosine pentaacetate

2-methoxyadenosne (spongosine)

Scheme 2

Claims

1. A method of synthesising a 2-substituted adenosine of formula I, which comprises: nitrating adenosine pentaacetate using tetrabutylammonium nitrate (TBAN) or tetramethylammonium nitrate (TMAN) to produce 2-nitroadenosine pentaacetate; reducing the amount of TBAN or TMAN contaminating the 2-nitroadenosine pentaacetate; and then producing the 2-substituted adenosine from the 2-nitroadenosine pentaacetate:



I

wherein R = C_{1-6} alkoxy (straight or branched), a phenoxy group (unsubstituted, or mono-, or di-substituted by halo, amino, CF_3-, cyano, nitro, C_{1-6} alkyl, or C_{1-6} alkoxy) or a benzoyl group (unsubstituted, or mono-, or di-substituted by halo, amino, CF_3-, cyano, nitro, C_{1-6} alkyl, or C_{1-6} alkoxy).

2. A method according to claim 1, wherein the amount of TBAN or TMAN contaminant is reduced by triturating the 2-nitroadenosine pentaacetate with isopropanol and washing the triturated 2-nitroadenosine pentaacetate with water.

3. A method according to claim 1 or 2, wherein the 2-substituted adenosine is produced from the 2-nitroadenosine pentaacetate by deprotecting the 2-nitroadenosine pentaacetate and reaction with a C_{1-6} alkoxide anion or a phenoxide anion.

4. A method according to any preceding claim, wherein the 2-substituted adenosine is 2-methoxy adenosine, and this is produced from the 2-nitroadenosine pentaacetate by reaction with methoxide anion from methanol/NaOMe, methanol/n-BuLi, methanol/NaOH, or methanol/NaH.

5. A method according to any preceding claim, which further comprises synthesising the adenosine pentaacetate by acylating adenosine.

6. A method according to claim 5, wherein the adenosine is acylated to form an O-tri-acetyl and/or tetra-acetyl derivative of adenosine, the derivative(s) is isolated, and the isolated derivative(s) is acylated to produce adenosine pentaacetate.

7. A method according to claim 5 or 6 which further comprises washing the adenosine pentaacetate to remove contaminating adenosine tetraacetate before nitrating the washed adenosine pentaacetate to form the 2-nitroadenosine pentaacetate.

8. A method of synthesising a 2-substituted adenosine of formula I, which comprises acylating adenosine to form an O-tri-acetyl and/or tetra-acetyl derivative of adenosine, isolating the derivative(s), acylating the isolated derivative(s) to produce adenosine pentaacetate, and producing the 2-substituted adenosine from the adenosine pentaacetate.

9. A method according to claim 8 which further comprises washing the adenosine pentaacetate to reduce the amount of contaminating adenosine tetraacetate before producing the 2-substituted adenosine from the washed adenosine pentaacetate.

10. A method of synthesising a 2-substituted adenosine of formula I, which comprises acylating adenosine, or an acylated derivative of adenosine, to form adenosine pentaacetate, washing the adenosine pentaacetate to reduce the amount of contaminating adenosine tetraacetate, and producing the 2-substituted adenosine from the washed adenosine pentaacetate.

11. A method according to any of claims 8 to 10, which further comprises nitrating the adenosine pentaacetate to produce 2-nitroadenosine pentaacetate, and producing the 2-substituted adenosine from the 2-nitroadenosine pentaacetate.

12. A method according to claim 11, wherein the 2-substituted adenosine is 2-methoxyadenosine, and is produced by reacting methoxide anion from methanol/NaOMe, methanol/n-BuLi, methanol/NaOH, or methanol/NaH with the 2-nitroadenosine pentaacetate.

13. A method according to any of claims 1, 2, or 11 which further comprises converting 2-nitroadenosine pentaacetate to 2-chloroadenosine pentaacetate before producing the 2-substituted adenosine from the 2-chloroadenosine pentaacetate.

14. A method of synthesising a 2-substituted adenosine, which comprises converting 2-chloroadenosine pentaacetate to the 2-substituted adenosine.

15. A method according to claim 14, which further comprises producing the 2-chloroadenosine pentaacetate from 2-nitroadenosine pentaacetate.

16. A method according to any of claims 13 to 15, wherein the 2-substituted adenosine is 2-methoxyadenosine, and the 2-chloroadenosine pentaacetate is converted to 2-methoxyadenosine by reaction with methoxide anion from methanol/NaOMe, methanol/n-BuLi, methanol/NaOH, or methanol/NaH with the 2-nitroadenosine pentaacetate.

17. A 2-substituted adenosine synthesised by a method according to any preceding claim.

18. A method of synthesising 2-methoxyadenosine, which comprises reacting methoxide anion from methanol/NaOMe, methanol/n-BuLi, methanol/NaOH, or methanol/NaH with the 2-nitroadenosine pentaacetate.

19. A method of synthesising 2-methoxyadenosine, which comprises the steps shown in scheme 1 or 2.

20. A method of synthesising 2-methoxyadenosine, which is substantially as described.

21. 2-methoxyadenosine which is >96% pure.

22. A method of synthesising 2-nitroadenosine pentaacetate, which comprises nitrating adenosine pentaacetate using TBAN or TMAN to produce 2-nitroadenosine pentaacetate, and reducing the amount of TBAN or TMAN contaminating the 2-nitroadenosine pentaacetate.

23. A method according to claim 22, wherein the amount of TBAN or TMAN contaminant is reduced by triturating the 2-nitroadenosine pentaacetate with isopropanol and washing the triturated 2-nitroadenosine pentaacetate with water.